

Claims 1-11, 16-27 and 32 have been canceled. Claims 33-63 have been newly added. The newly added claims find support throughout the specification. In particular, claims 33-37 and 41-46 find support on pages 12-23 and Figure 4 of the specification; claims 38-40 and 47- 63 are particularly supported by the disclosure found on page 23 of the specification. No new matter has been added by these claim.

**Response to the objections to the drawings**

The drawings filed January 28, 2002 were objected to for the reasons indicated on the form PTO 948. New drawings are concurrently submitted herewith in order to obviate these objections.

**Response to the notice to comply with sequence rules**

The Examiner contends that sequence identifiers are missing from the specification and that the application fails to comply with 37 C.F.R. 1.821 through 1.85. Applicants have amended the specification to include sequence identifiers where appropriate and submit concurrently herewith a substitute sequence listing.

**Response to the Examiner's withdrawal of claims**

The Examiner contends that claim 3, 9 and 10 are drawn to an independent and distinct invention. Applicants respectfully disagree. However, in the interest of furthering prosecution claims 3, 9 and 10 have been canceled. Applicants hereby reserve their right to pursue these claims in the future.

**Response to the rejections under 35 U.S.C. 112 (first paragraph)**

Claims 1-2, 4-8, 11, 16-27 and 32 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while enabling for nucleic acids of SEQ ID NO: 28 and nucleic acids encoding SEQ ID NO: 29, certain methods of using those nucleic acids and plants transformed with those nucleic acids purportedly does not provide reasonable enablement for other methods of using those nucleic acids, nor for nucleic acids encoding portions of SEQ ID NO: 29 or that hybridize to SEQ ID NO 28 under conditions of unspecified stringency or that have 200 base

pair regions with 88% identity to SEQ ID NO: 28, methods of using those nucleic acids, and plants transformed with those nucleic acids.

The Examiner contends that the specification does not provide the guidance that one of ordinary skill in the art would need in order to practice the invention of claims 1-2, 4-8, 11, 16-27 and 32. In particular, the Examiner alleges that there is no guidance provided for making amino acid substitutions to produce the polypeptides of the instant invention.

Claims 1-2, 4-8, 11, 16-27 and 32 have been canceled. Thus, this rejection will be addressed as it applies to newly added claims 33-63. Claims 33-63 are directed to nucleic acids from cassava which encode polypeptides having starch branching enzyme II activity (SBEII), methods of using these nucleic acids and plants and plant cells comprising these nucleic acids. The specification clearly provides the proper guidance that one of skill in the art would need in order to practice the invention as claimed. In particular, the specification of the instant application teaches the discovery of novel genes from cassava that encode polypeptides having starch branching enzyme II activity (see pages 12-23). These enzymes are critical in the starch biosynthetic pathway. The specification explicitly teaches a method that one could use in order to isolate SBEII genes from cassava. The specification also clearly teaches that nucleic acid sequence SEQ ID NO: 28 encodes the polypeptide of SEQ ID NO: 29 which has SBEII activity (see pages 12-23 and Figure 4). Thus, one of skill in the art would be able to practice the claimed invention without any guidance other than that which is taught in the specification. Furthermore, the degenerative nature of the genetic code is well documented in the art. One of skill in the art would know that, given the degenerative nature of the genetic code, there can be a significant amount of variation in individual sequences that encode identical polypeptides. Thus, one of skill in the art would see that the Applicants have disclosed a nucleic acid encoding a particular polypeptide sequence and also the variations of this nucleic acid sequence that are a result of the degenerative nature of the genetic code.

With regards to the Examiner's concerns regarding the methods of the instant invention, attached herewith is a Declaration under 37 C.F.R. 1.132 by Joseph Emling which describes the experimental evidence demonstrating that the methods of the instant claims can be used to produce starch with altered properties. In particular, the Declaration describes the results of two replications (A and B) of a viscosity test of transformed 'line 9.18', which comprises an SBEII

gene in antisense orientation, and a control. As shown in the attached Exhibit B, 'line 9.18' had a 20% increase in peak viscosity and a 50% increase in hot paste viscosity as compared to the control. These results clearly demonstrate that the transformation of plants with an SBEII gene in antisense orientation results in the production of starch with altered properties.

Given the description provided by the specification and the state of the prior art, one of skill in the art would be able to practice the invention as claimed without further guidance. As such, one skilled in the art at the time of filing would be able to make and use the invention of the instant claims.

Claims 1-2, 4-8, 11, 16-27 and 32 stand rejected under 35 U.S.C. 112, first paragraph for purportedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention. The Examiner contends that the structural features of all the nucleic acids that encode an "effective portion" of SEQ NO: 29 or that have 200 bp and 88% identity to SEQ ID NO: 28 have not been adequately described.

Claims 1-2, 4-8, 11, 16-27 and 32 have been canceled. Thus, this rejection will be addressed as it applies to newly added claims 33-63.

As stated above, claims 33-63 are directed to nucleic acids encoding polypeptides having starch branching enzyme II activity, methods of using these nucleic acids and plants and plant cells comprising these nucleic acids. The specification provides the proper guidance that one of skill in the art would need in order to practice the invention as claimed and clearly describes the nucleic acids of the instant invention in such detail that one of skill in the art would readily comprehend that the Applicants were in possession of the claimed invention at the time the instant application was filed. In particular, the specification of the instant application teaches the discovery of multiple novel genes encoding polypeptides with starch branching enzyme II activity (see pages 12-23) which are critical in the starch biosynthetic pathway. The currently pending claims are directed to these nucleic acid sequences. The specification clearly teaches nucleic acid sequence SEQ ID NO: 28 which encodes the polypeptide of SEQ ID NO: 29 which has SBEII activity (see pages 12-23 and Figure 4). Furthermore, with regards to the Examiners objection to the term "effective portion", on page 3 of the specification there is an explicit definition of the term "effective portion". Upon reading this definition, one of skill in the art

would recognize the meaning of the term "effective portion" as it is used in the claims. Thus, one of skill in the art would fully understand that the Applicants were in possession of the claimed invention at the time the application was filed and that it is fully described in the specification. As such, the Applicant respectfully requests that this rejection under 35 U.S.C. 112, first paragraph be withdrawn.

**Response to the rejection under 35 U.S.C. 112 (second paragraph)**

Claim 1-2, 4-8, 11, 16-27 and 32 stand rejected under 35 U.S.C. 112, second paragraph, as purportedly being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention.

Claim 1-2, 4-8, 11, 16-27 and 32 have been canceled and as such the rejections will be addressed as they pertain to newly added claims 33-63.

The Examiner asserts that the term "an effective portion" as it appears in claim 1 is indefinite. Applicants respectfully disagree with this assertion and again direct the Examiner to page 3 of the specification where this term is fully defined. Upon reading this definition one of skill in that art would fully understand the metes and bounds of the claimed invention.

The Examiner asserts that the term "functionally equivalent nucleotide sequence" is indefinite. Newly added claims 33-63 do not include this term. As such, this rejection is moot.

The Examiner asserts that the term "stringent hybridization conditions" is indefinite. Newly added claims 33-63 do not include this term. As such, this rejection is moot.

**Response to the rejection under 35 U.S.C. 102(b)**

Claims 1-2, 4 and 11 stand rejected under 35 U.S.C. 102(b) as being anticipated by Fisher *et al.*

Claims 1-2, 4 and 11 have been canceled. As such, this rejection will be addressed as it applies to newly added claims 33-63.

Newly added claims 33-63 are directed to nucleic acid sequences from cassava encoding polypeptides having starch branching enzyme activity and having the amino acid sequence of SEQ ID NO: 29; nucleic acid sequences from cassava encoding polypeptides having starch branching enzyme activity and having at least 88% sequence identity to SEQ ID NO: 28;

methods of using these nucleotide sequences and plants and plant cells comprising these sequences.

Fisher *et al.* teach a nucleic acid isolated **from maize** which encodes a polypeptide having SBEII activity. Fisher *et al.* do not teach a nucleic acid sequence isolated **from cassava** having at least 88% sequence identity to SEQ ID NO: 28 or a nucleic acid sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 29 and having SBEII activity. As such Fisher *et al.* does not anticipate the present claims.

### Conclusion

The foregoing amendments and remarks are being made to place the application in condition for allowance. Applicants respectfully request reconsideration and the timely allowance of the pending claims. A favorable action is awaited. Should the Examiner find that an interview would be helpful to further prosecution of this application, he is invited to telephone the undersigned at his convenience.


If there are any additional fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-0310. If a fee is required for an extension of time under 37 C.F.R. 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE****In the specification:**

The paragraph beginning on page 4, line 12 has been replaced with the following paragraph:

Conveniently the nucleic acid sequence is obtainable from cassava, preferably obtained therefrom, and typically encodes a polypeptide obtainable from cassava. In a particular embodiment, the encoded polypeptide may have the amino acid sequence NSKH (**SEQ ID NO: 32**) at about position 697 (in relation to Figure 4 (SEQ. ID. NO. 29)), which sequence appears peculiar to an isoform of the SBE class A enzyme of cassava, other class A SBE enzymes having the conserved sequence DA D/E Y (**SEQ ID NO: 33**) (Burton et al., 1995 cited above).

The paragraph beginning on page 6, line 7 has been replaced with the following paragraph:

In another aspect the invention provides a polypeptide having SBE activity, the polypeptide comprising an effective portion of the amino acid sequence shown in Figure 4 (SEQ. ID. NO. 29) or Figure 13 (SEQ. ID. NO. 31). The polypeptide is conveniently one obtainable from cassava, although it may be derived using recombinant DNA techniques. The polypeptide is preferably in substantial isolation from other polypeptides of plant origin, and more preferably in substantial isolation from any other polypeptides. The polypeptide may have amino acid residues NSKH (**SEQ ID NO: 32**) at about position 697 (in the sequence shown in Figure 4 (SEQ. ID. NO. 29)), instead of the sequence DA D/E Y (**SEQ ID NO: 33**) found in other SBE class A polypeptides. The polypeptide may be used in a method of modifying starch *in vitro*, the method comprising treating starch under suitable conditions (of temperature, pH etc.) with an effective amount of the polypeptide.

The paragraph beginning on page 17, line 19 has been replaced with the following paragraph:

To confirm this a primer (CSBE218, SEQ. ID. NO. 19) was made to a region in the 3' UTR (untranslated region) of pSJ101 and used in combination with CSBE214 (SEQ. ID. NO. 15) primer to recover by PCR a full length cDNA from both leaf and root cDNA. These clones

were sequenced and designated pSJ106 & pSJ107 respectively. The sequence and predicted ORF of pSJ107 is shown in Figure 4 (SEQ. ID. NO. 28). The long ORF in plasmid pSJ106 was found to be interrupted by a stop codon (presumably introduced in the PCR process) approximately 1 kb from the 3' end of the gene, therefore another cDNA clone (designated pSJ116) was amplified in a separate reaction, cloned and sequenced. This clone had an intact ORF (data not shown).

There were only a few differences in these two sequences (in the transit peptide aa 27-41: YRRTSSCLSFNFKEA (SEQ ID NO: 34) to DRRTSSCLSFIFKKAA (SEQ ID NO: 35) and L831 in pSJ107 to V in pSJ116 respectively).

The paragraph beginning on page 19, line 17 has been replaced with the following paragraph:

A comparison of all known SBE II protein sequences shows that the cassava SBE II gene is most closely related to the pea gene (Figure 8). The two proteins are 86.3% identical over a 686 amino acid range which extends from the triple proline "elbow" (Burton *et al.*, 1995 Plant J. 7, 3-15) to the conserved VVYA (SEQ ID NO: 36) sequence immediately preceding the C-terminal extensions (data not shown). All SBE II proteins are conserved over this range in that they are at least 80% similar to each other. Remarkably however, the sequence conservation between the pea, potato and cassava SBE II proteins also extends to the N-terminal transit peptide, especially the first 12 amino acids of the precursor protein and the region surrounding the mature terminus of the pea protein (AKFSRDS (SEQ ID NO: 37)). Because the proteins are so similar around this region it can be predicted that the mature terminus of the cassava SBE II protein is likely to be GKSSHES (SEQ ID NO: 38). The precursor has a predicted molecular mass of 96 kD and the mature protein a predicted molecule mass of 91.3 kD. The cassava SBE II has a short acidic tail at the C-terminal although this is not as long or as acidic as that found in the pea or potato proteins. The significance of this acidic tail, if any, remains to be determined. One notable difference between the amino acid sequence of cassava SBE II and all other SBE II proteins is the presence of the sequence NSKH (SEQ ID NO: 32) at around position 697 instead of the conserved sequence DAD/EY (SEQ ID NO: 33). Although this conserved region forms

part of a predicted  $\alpha$ -helix (number 8) of the catalytic ( $\beta/\alpha$ )<sub>8</sub> barrel domain (Burton et al 1995 cited previously), this difference does not abolish the SBE activity of the cassava protein as this gene can still complement the glycogen branching deletion mutant of *E. coli*. It may however affect the specificity of the protein. An interesting point is that the other cassava SBE II clone pSJ94 has the conserved sequence DADY.